NTP
National Toxicology Program

Aging and Disease Phenotyping in Multiple Inbred Mouse Strains

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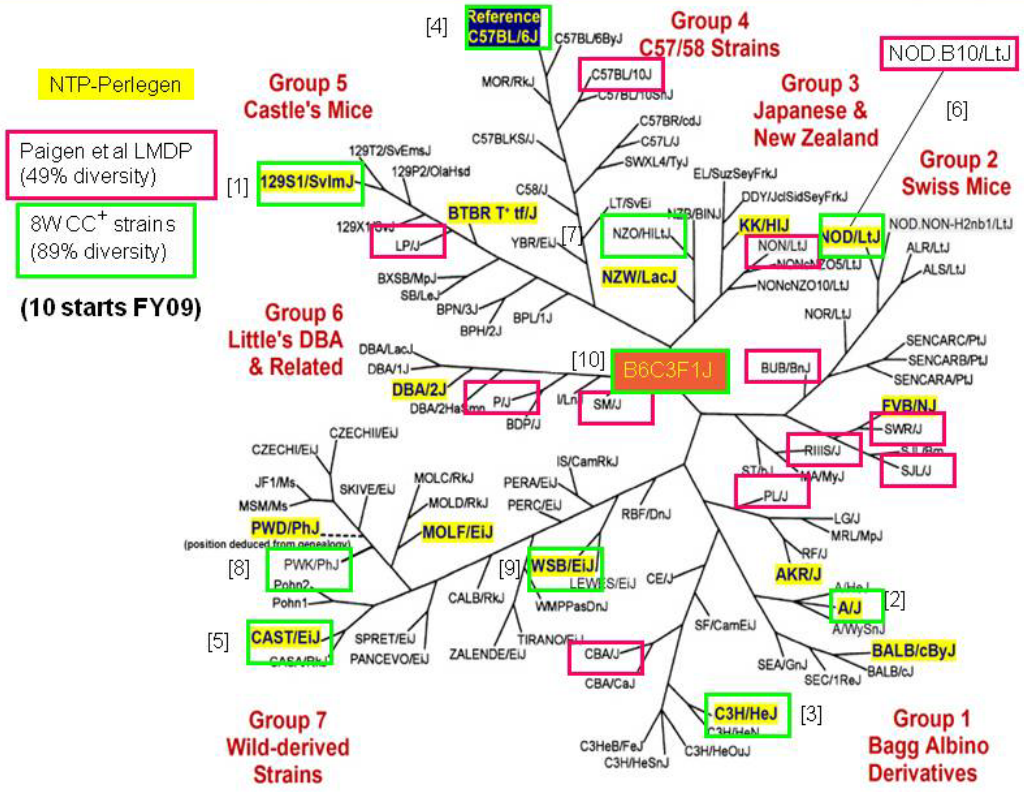
NTP Board of Scientific Counselors
December 9-10, 2009

Background and Rationale

- Individual genetic and epigenetic differences within the human population are believed to be the basis for individual susceptibility to environmental stressors, including idiosyncratic drug toxicities.
- Safety assessments are conducted with a small number of commonly used animal models with limited genetic diversity that is insufficient to evaluate the influence of individual genetic differences on chemical and drug toxicity, and to extrapolate to human toxicity and disease.
- To improve extrapolation of results from mouse models for human hazard identification and characterization, a benchmark reference data set derived across multiple strains of mice is critical.
- Benchmark reference data base would aid selection of the most appropriate strains for study and the extrapolation of results between species.


NTP-Perlegen
Paigen et al LMDP
(49% diversity)
8W CC+ strains
(89% diversity)
(10 starts FY09)
Group 5
Castle's Mice
Group 4
C57/58 Strains
Group 3
Japanese &
New Zealand
Group 2
Swiss Mice
Group 6
Little's DBA
& Related
Group 7
Wild-derived
Strains
Group 1
Bagg Albino
Derivatives
[1]
[2]
[3]
[4]
[5]
[6]
[7]
[8]
[9]
[10]
Reference
C57BL/6J
C57BL/6ByJ
C57BL/10J
C57BL/10SnJ
MOR/RkJ
C57BR/cdJ
C57L/J
C57BLKS/J
SWXL4/TyJ
129T2/SvEmsJ
129P2/OlaHsd
129S1/SvImJ
C58/J
BTBR T+ tf/J
LP/J
129X1/SvJ
LT/SvEi
NZO/HlLtJ
NZB/BINJ
EL/SuzSeyFrkJ
DDY/JdSidSeyFrkJ
KK/HlJ
NOD/LtJ
NOD.B10/LtJ
NOD.NON-H2nb1/LtJ
NON/LtJ
NONcNZO5/LtJ
NONcNZO10/LtJ
ALR/LtJ
ALS/LtJ
NZW/LacJ
YBR/EiJ
BXSB/MpJ
SB/LeJ
BPN/3J
BPL/1J
BPH/2J
NOR/LtJ
SENCARC/PtJ
SENCARB/PtJ
SENCARA/PtJ
DBA/LacJ
DBA/1J
DBA/2J
DBA/2HaSmn
P/J
BDP/J
I/LnJ
SM/J
B6C3F1J
BUB/BnJ
FVB/NJ
SWR/J
SJL/J
RIIIS/J
ST/bJ
MA/MyJ
PL/J
LG/J
MRL/MpJ
RF/J
AKR/J
A/J
A/WySnJ
BALB/cByJ
BALB/cJ
SEA/GnJ
SEC/1ReJ
C3H/HeJ
C3H/HeOuJ
C3HeB/FeJ
C3H/HeSnJ
CBA/J
CBA/CaJ
SF/CamEiJ
CE/J
RBF/DnJ
IS/CamRkJ
PERA/EiJ
PERC/EiJ
WSB/EiJ
LEWES/EiJ
WMPPasDnJ
CALB/RkJ
TIRANO/EiJ
ZALENDE/EiJ
CZECHII/EiJ
CZECHI/EiJ
JF1/Ms
MSM/Ms
SKIVE/EiJ
MOLC/RkJ
MOLD/RkJ
MOLF/EiJ
PWD/PhJ
(position deduced from genealogy)
PWK/PhJ
Pohn2
Pohn1
CAST/EiJ
CASA/RkJ
SPRET/EiJ
PANCEVO/EiJ

Aging Cohort Study in 10 mouse strains (see below)

Name	CASRN	Study No.
Aging Cohort Study –129/SvlmJ mouse	MOUSEPHENO1	C09045
Aging Cohort Study - A/J mouse	MOUSEPHENO2	C09046
Aging Cohort Study - C3H/HeJ mouse	MOUSEPHENO3	C09047
Aging Cohort Study - C57/BL/6J mouse	MOUSEPHENO4	C09048
Aging Cohort Study - CAST/EiJ mouse	MOUSEPHENO5	C09049
Aging Cohort Study - B6C3F1J mouse	MOUSEPHENO6	C09050
Aging Cohort Study - NOD.B10^{H2b}/LtJ mouse	MOUSEPHENO7	C09051
Aging Cohort Study - PWK/PhJ mouse	MOUSEPHENO8	C09052
Aging Cohort Study - WSB/EIJ mouse	MOUSEPHENO9	C09053
Aging Cohort Study - NZO/HiLtJ mouse	MOUSEPHENO10	C09054

Aging & Disease Phenotypes

- Bev Paigen et al. The Jackson Labs
- 32 inbred strains
- Cohorts for aging & phenotyping
- 32 male and 32 female adult mice/strain for survival analysis (4 per HEPA filtered positive pressure air cage with white pine shavings and acidified water (pH 3)
- 45 male and 45 female/strain interim sacs at **26, 52, & 78 wk** intervals for molecular analysis
- 2nd cohort of 32 females for age related reproduction
- Introduced staggered cohorts at 3 month intervals
- **SPF (4X/yr) for 15 viruses and 17 bacterial species**

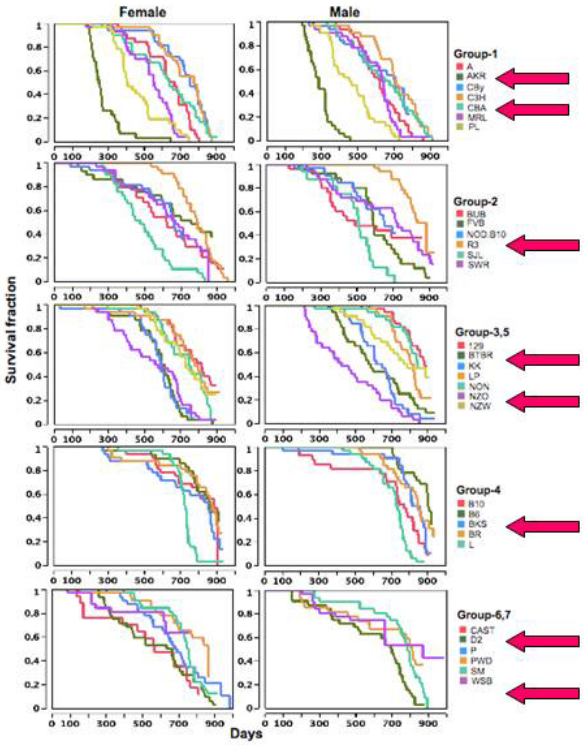
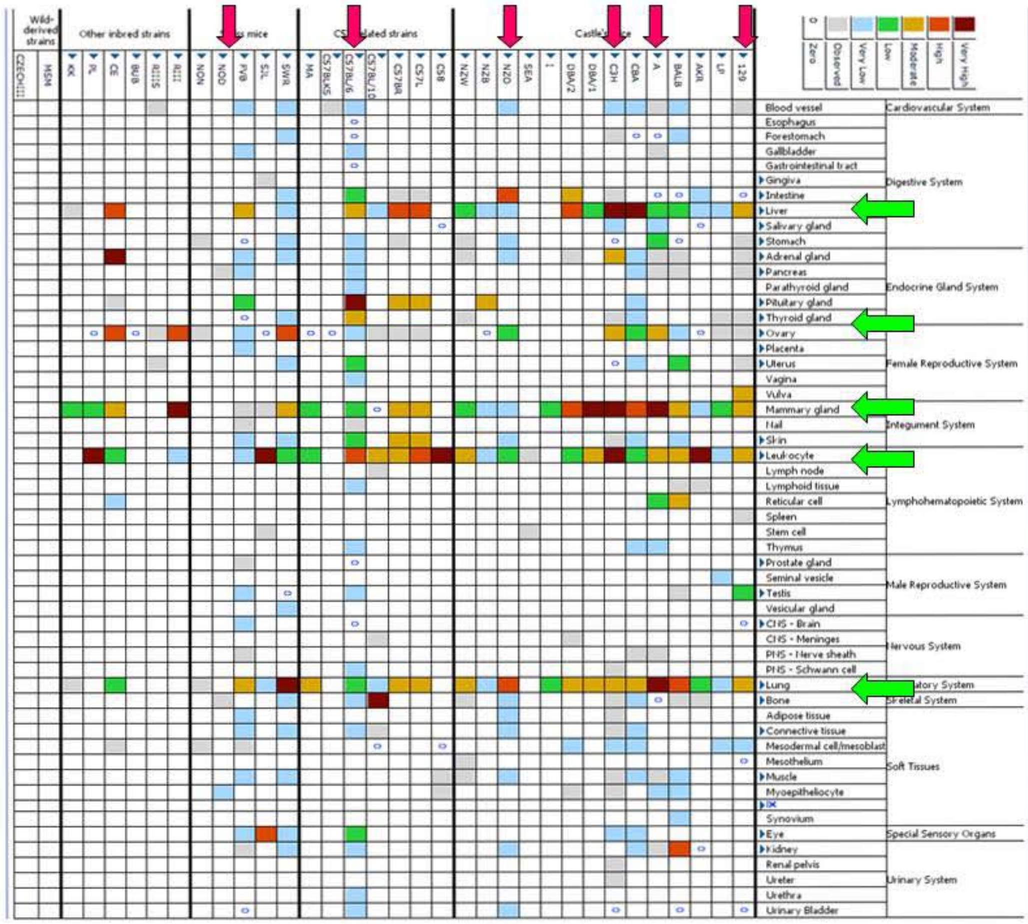


Figure 2. Survival curves were generated by Kaplan Meier method


Wild-derived strains
Other inbred strains
Castle's mice
CZECHII
MSM
KK
PL
CE
BUB
RIIIS
RII
NON
NOD
FVB
SJL
SWR
MA
C57BLKS
C57BL/6
C57BL/10
C57BR
C57L
C58
NZW
NZB
NZO
SEA
I
DBA/2
DBA/1
C3H
CBA
A
BALB
AKR
LP
129
Zero
Observed
Very Low
Low
Moderate
High
Very High
Blood vessel
Esophagus
Forestomach
Gallbladder
Gastrointestinal tract
Gingiva
Intestine
Liver
Salivary gland
Stomach
Adrenal gland
Pancreas
Parathyroid gland
Pituitary gland
Thyroid gland
Ovary
Placenta
Uterus
Vagina
Vulva
Mammary gland
Nail
Skin
Leukocyte
Lymph node
Lymphoid tissue
Reticular cell
Spleen
Stem cell
Thymus
Prostate gland
Seminal vesicle
Testis
Vesicular gland
CNS - Brain
CNS - Meninges
PNS - Nerve sheath
PNS - Schwann cell
Lung
Bone
Adipose tissue
Connective tissue
Mesodermal cell/mesoblast
Mesothelium
Muscle
Myoepitheliocyte
Synovium
Eye
Kidney
Renal pelvis
Ureter
Urethra
Urinary Bladder
Cardiovascular System
Digestive System
Endocrine Gland System
Female Reproductive System
Integument System
Lymphohematopoietic System
Male Reproductive System
Nervous System
Skeletal System
Soft Tissues
Special Sensory Organs
Urinary System

Key Issues

- Selection of appropriate mouse strains as models for human genetic diversity, environmental exposures, and disease susceptibility
- Study design and conduct used for detecting variable ranges of response to sporadic or spontaneous disease and xenobiotic exposure to multiple strains of inbred mice to model potential xenobiotic induced human disease in a GEI paradigm of human relevance.

Aims

1. Determine survival and sporadic disease incidence under NTP study study conditions for 10 selected strains (**bench mark reference population of inbred strains; captures 90% known SNP diversity**).

2. Collect appropriate data and tissue/fluids for archival and analyze, if warranted, **to determine association between haplotype and pre-disease phenotypes and disease outcome (use for strain selection for variable responses)** and **to identify biomarkers for exposure & effect**

3. Perform quantitative trait analysis (limited) **to determine the highly penetrant genetic variant differences between strains that segregate with disease phenotypes**.

4. Perform a comparative genetic, histogenetic pathology, and genomic analysis between between strains and **identify possible orthologs with potential functional linkage (MOA) to aid in extrapolation between the mouse and human**.

5. Prepare for multiple inbred strain and diverse outbred stock for prechronic and chronic toxicology and disease studies.

Components

- Age Related Survival and Spontaneous Disease Phenotypes in Selected Genetically Diverse Inbred Laboratory Mice (20% survival)
- Age Related Biomarkers (Serum Chemistry and Hematology) and Genetical Genomics (26, 52, and 78 weeks)
- Age Related Functional Phenotypes of the Cardiovascular and Respiratory Systems Genomics (26, 52, and 78 weeks)

Aging & Disease Phenotyping Study Design

- **Diet:** NTP 2000 started at day of receipt
- **Environment and Animal Husbandry**
- **Housing:** - 1 ♂or 4 or 5♀ per cage (size dependent).
- **Infectious Disease Surveillance**
- **Cage observations** (2x/day) for age related behavior
- Cohorts loaded independently and all mice shall be received for each cohort at the same time
- All aspects of the study to be conducted in accord with the NTP Specifications

Continued..

Cohort 1: Age Related Survival and Spontaneous Disease Phenotypes in Selected Genetically Diverse Inbred Laboratory Mice (20% survival)

- Survival and age-related disease phenotype and prevalence (until survival for that sex/strain is reduced to 20% or 110 weeks of age

1) The survival cohort of 115 mice/sex/strain shall be maintained until survival for that sex/strain is reduced to 20% or 110 weeks of age (115 mice x 2 sexes; 230 mice/sex/strain)

2) A complete necropsy and a set of tissues shall be collected from each natural death, moribund mouse, or mouse euthanized at study termination and processed for histopathologic examination as described in the Specifications.

3) Statistical Analysis (for age-related survival and tumor phenotype and prevalence differences across strains; Kaplan-Meier; poly-k, and poly-3, as well as analysis of continuous variables, etc.)

Continued..

Cohort 2: Age Related Biomarkers (Serum Chemistry and Hematology), Metabolomics, and Genetical Genomics Genomics (26, 52, and 78 weeks)

1. Age related interim evaluation for histopathology, clinical pathology (chemistry and hematology) measurement at 26, 52, and 78 weeks of age (24/sex/age/strain x 3 ages x 2 sexes = 144 mice total/strain). This study will be concluded at 78 wks of age.
2. Tissue/Organ specific histopathology – NTP Specifications
3. Blood and Bone Marrow for hematology and plasma for clinical chemistry and identification of pre-disease biomarkers
4. Tissue archival: Specimens (50 mg pcs; **all masses, plus heart, liver, lung, kidney, ovary, testes, mammary gland, bone marrow (femur) as protocol specifies**; removed within 5 min and flash frozen in LN2; stored at -80°C for RNA (transcript profiles) & DNA (SNP, CNV, and methylation screen for epigenetics) for retrospective analysis after target tissues identified by histology
5. Plasma (archival) for metabolomics and additional biomarkers

Continued..

Cohort 3: **Age Related Functional Phenotypes of the Cardiovascular and Respiratory Systems (26, 52, and 78 wks of age)**

- Cardiac and respiratory function will be assessed at two different times (7 mice x 2 sexes x 3 ages x 2 phenotypes; 84 mice/strain).
- Between 26-30, 48-52, and 74-78 wks of age, cardiac function will be monitored using a surgically implanted PhysioTel PA-C10 transmitter (Data Sciences International, St. Paul, MN) to measure arterial blood pressure (systolic, diastolic and mean) and pulse rate.
- Another independent set of mice will assessed at 28, 52, and 78 wks of age pulmonary function measurements using a flexiVent data acquisition system (v5.1, SCIREQ Electronics; Montreal, Quebec, Canada).
- Acomplete set of tissues collected and processed for histopathologic examination of mice that present in a moribund condition during evaluation or are euthanized at study termination for correlation with cohort 1 and 2 studies.

Summary/Conclusions

- Improve hazard identification and characterization and across species extrapolation
- Move from observational to predictive models
- Identify resistant and sensitive models for predicting environmental hazards (Reduce type1 and type 2 error rates)
- Identifies gene-environment interactions
- Determine common mechanisms in disease processes across species to aid functional validation of causally related genes and biological pathways

Status: In progress

- Cohort 1 – Aging and Spontaneous Disease Phenotyping initiated in 10 inbred strains
- Cohorts 2 & 3 have been approved by the PRC and under review by the Protocol Approval Committee

Acknowledgements

NTP/NIEHS

- Frank Johnson
- Rick Irwin
- John Bucher
- Keith Shockley
- Mike Cunningham
- Grace Kissling
- Molly Vallant
- Greg Travlos
- June Dunnick
- Project Review Committee
- Protocol Approval Committee

The Jackson Laboratory

- Bev Paigen
- Cat Lutz
- Gary Churchill

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Questions/Comments